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LEUCOCYTOZOON CAULLERYI

in

CHICKENS

(History and Diagnosis)

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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LEUCOCYTOZOON CAULLERYI IN CHICKENS

(History and Diagnosis)[1]

INTRODUCTION

Within recent years several serious outbreaks of acute leucocytozoonosis in fowls have been reported from Japan (4),[2] Taiwan (20), Ceylon (26), Thailand (4), Viet Nam (2), Burma, and Egypt (15). It is quite probable that the disease reported from Thailand as Bangkok Haemorrhagic Disease (12) is leucocytozoonosis (4). In Japan and Burma, the causative agent has been identified as Leucocytozoon caulleryi (14).

Leucocytozoonosis has been known for a considerable number of years and appears to be on the increase in South East Asia. It is capable of causing very heavy losses in poultry and could become a disease of considerable economic importance elsewhere. If introduced into the United States, the disease could cause serious losses to the poultry industry (15).

Leucocytozoon was reported in chickens in South Carolina in 1950. This is the first known report of any Leucocytozoon in chickens in North America (6). The organism was later designated as L. andrewsi (7) and is apparently of little significance.

The importance of leucocytozoonosis as an "emerging disease" especially in the Far East is obvious. It is suggested that all veterinary services maintain a careful watch for the appearance of this disease in their respective areas.

LIFE HISTORY OF LEUCOCYTOZOON

The genus Leucocytozoon is difficult to describe, but it is fundamentally very closely related to Plasmodium and Haemoproteus. Fertilization and sporogomy take place in a blood-sucking dipterous intermediate host very much as they do in the true malarial parasites (10, 19).

The life cycle of L. caulleryi is shown diagramatically in figure 1.

Macrogametocytes and microgametocytes are ingested by blood sucking Culicoides as they feed upon infected birds. The zygote formed becomes an elongated and motile ookinete. Oocysts

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[2] Underscored numbers in parentheses refer to Selected References at end of this report.

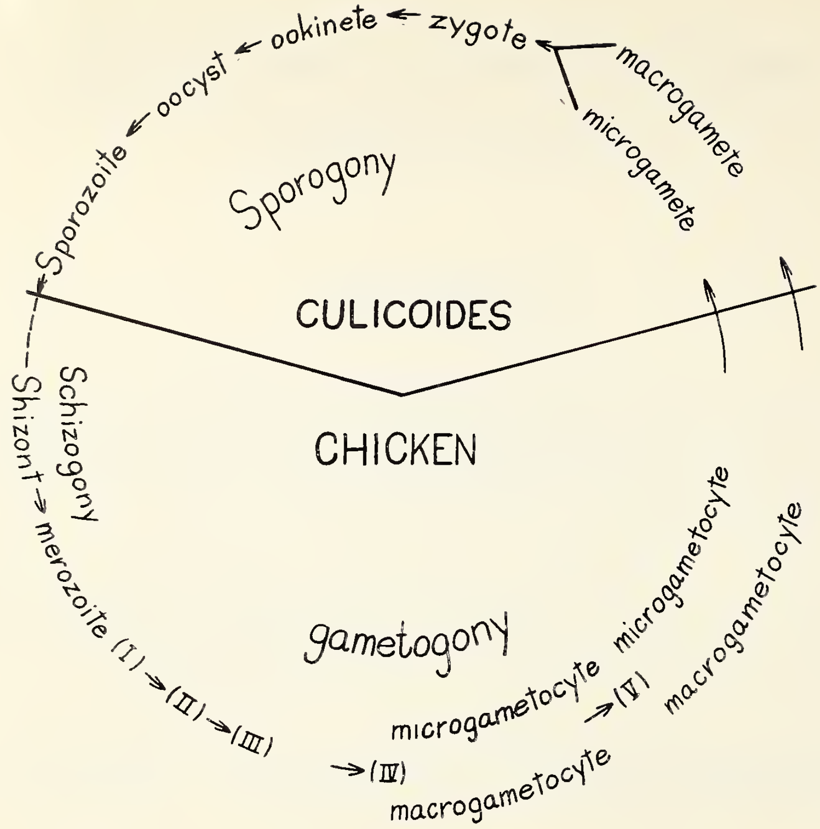

Figure 1.--Life cycle of L. caulleryi. (From the work of K. Akiba).

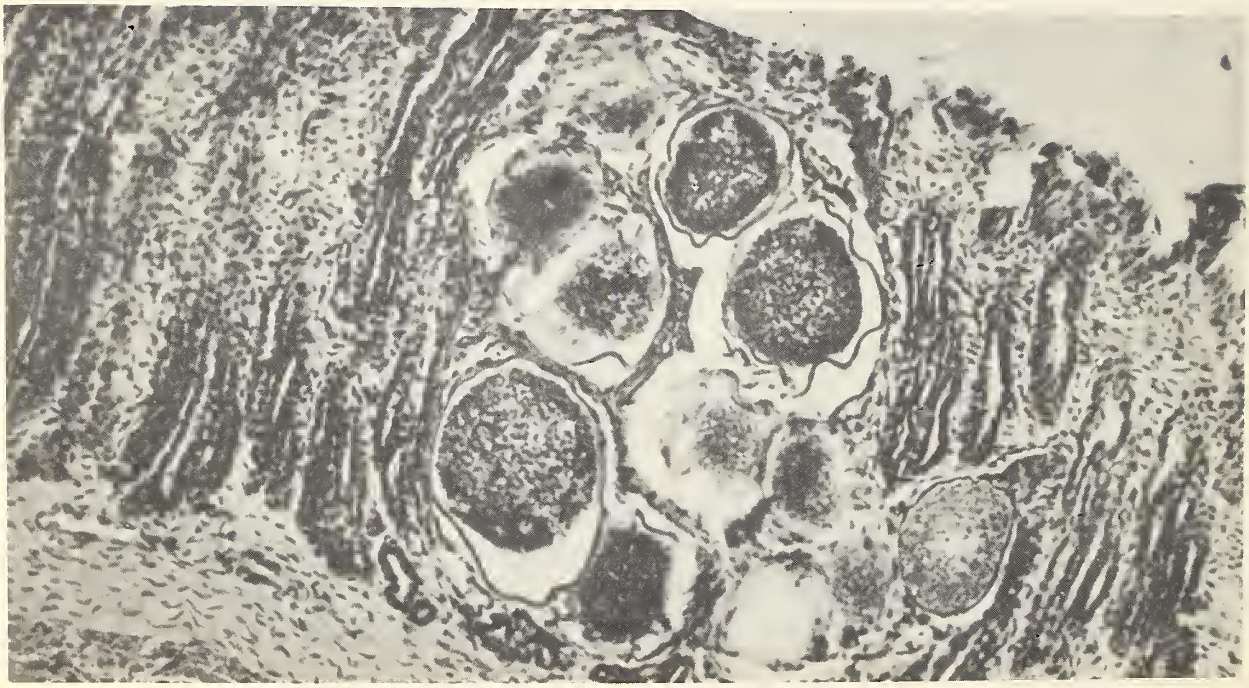

Figure 2.--Schizonts in lining of chicken gizzard.

are formed, develop to maturity, and release sporozites that are then transmitted by the *Culicoides* into susceptible birds (20). The sporozoites invade the endothelial cells of the capillaries and form schizonts, which ultimately mature and produce merozoites (22) (fig. 2). This starts the gametogony phase, which, according to Pan (22), is found either in the peripheral blood or in the tissue capillaries. Host cells are either the mature erythrocytes or erythroblasts.

Pan has described the process of gametogony in five stages:

Stage I. The merozoite is still free in the blood plasma (fig. 3).

Stage II. The parasite has invaded the erythrocyte or erythroblast (fig. 4). The size of the parasite is about the same as that of the free merozoite in the blood plasma.

Stage III. The parasite has grown and is larger than in stage II but is not sexually differentiated. This stage either is included within the host cells or is free from them. The size of the nearly mature parasite is approximately 11.8 X 9.2 μ (microns) the size of the host cell is approximately 15.1 X 13.6 μ.

Stage IV. The gametocyte may still be or may not be in the host cells, and differentiation of the two gametocytes is possible. The host cell is approximately 20.9 X 17.1 μ. The parasite is approximately 15.1 X 13.6 μ (figs. 5 and 6).

Stage V. One type of the gametocyte is still included in the host cell, but the nucleus of the host cell is lost. The gametocyte of the other type is free from the host cell, and the round or ovoid macrogametocyte or microgametocytes are found in the blood plasma (22) (fig. 7). They are then ready to repeat the schizogonous phase of the cycle.

INTERMEDIATE HOSTS

Work in Japan has shown that *Leucocytozoon caulleryi* is transmitted by the ceratopogonid fly *Culicoides arakawae* (2). This species of fly is also an important pest of poultry in lower Burma, but transmission by the fly has not been proved. However, epizootilogical evidence suggests that the fly may be a vector (15).

Culicoides arakawae has not been identified in North America. No species of *Culicoides* is known to transmit *Leucocytozoon* in Canada (18) or in the United States. However, several species of *Culicoides* in Canada have been found to be vectors of *Haemoproteus*.

The Simuliidae (black flies) are known to transmit *Leucocytozoon* infections in turkeys and ducks in the United States and Canada (18, 25). It is not known if the Simuliidae is the only intermediate host of *Leucocytozoon* in North America, or if it will act as an intermediate host for *L. caulleryi*.

According to reports in Canada, innumerable mosquitoes and flies of the genus *Stomoxys* were present on the turkey premises infected with *L. smithi*. It was suggested that flies other than the black fly may act as an intermediate host of *Leucocytozoon* (24).

Culex tarsalis, *C. stigmatosoma*, and *C. quinquefasciatus* have been incriminated as natural vectors of avian malaria. Previous studies on the feeding habits of these species of mosquitoes revealed that each fed frequently on avian hosts (23).

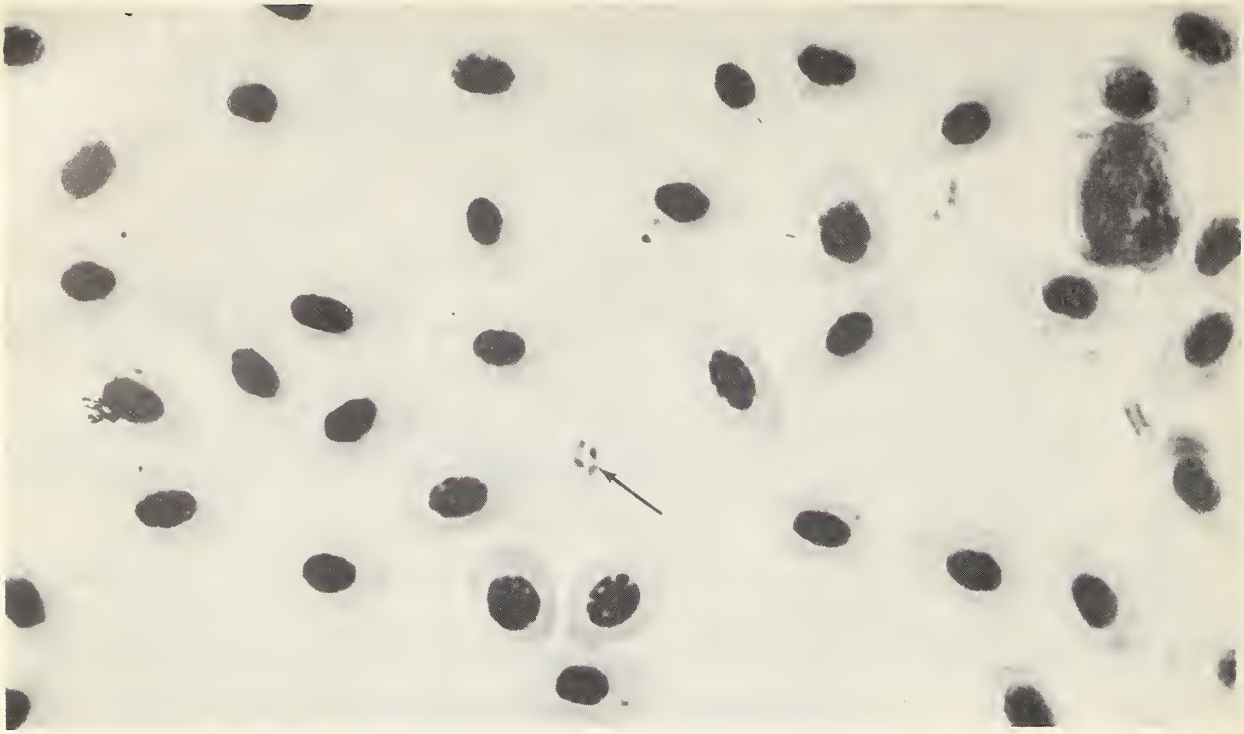

Figure 3.--Stage I: Merozoites free in the blood plasma. (Bloodsmear).

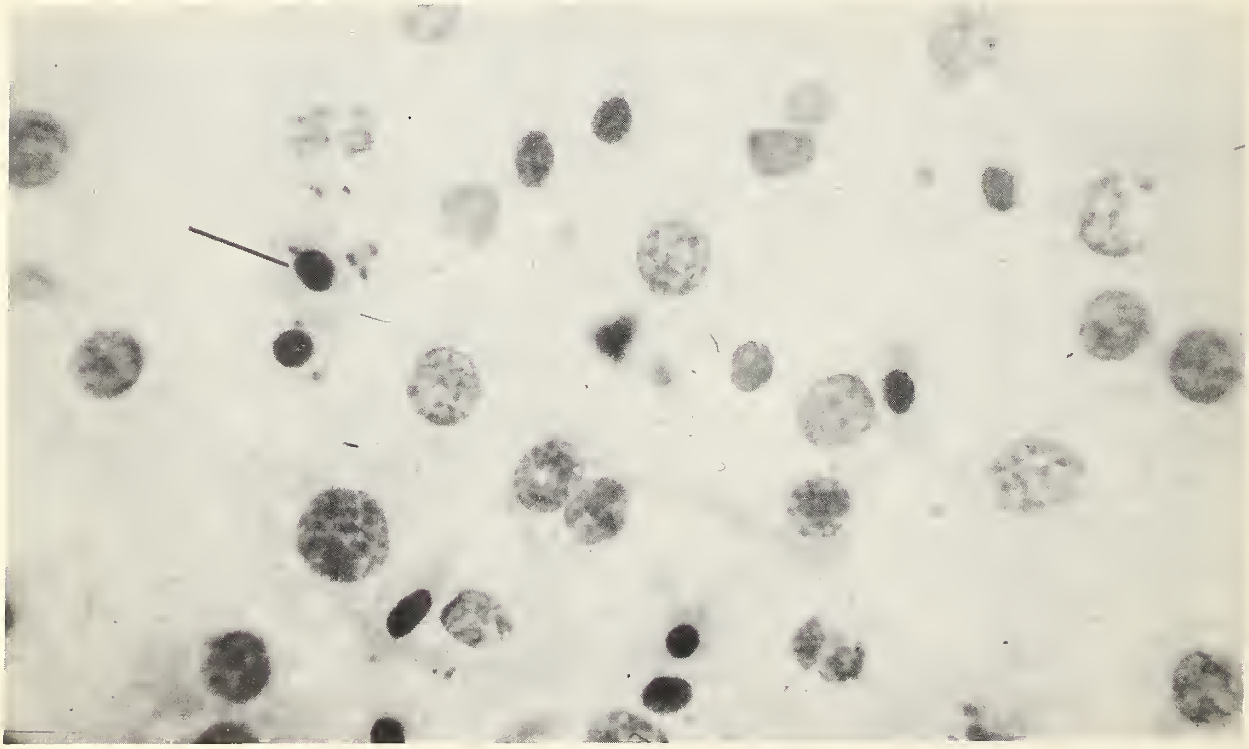

Figure 4.--Stage II: Merozoites have entered host erythrocite. (Stamp smear of the liver).

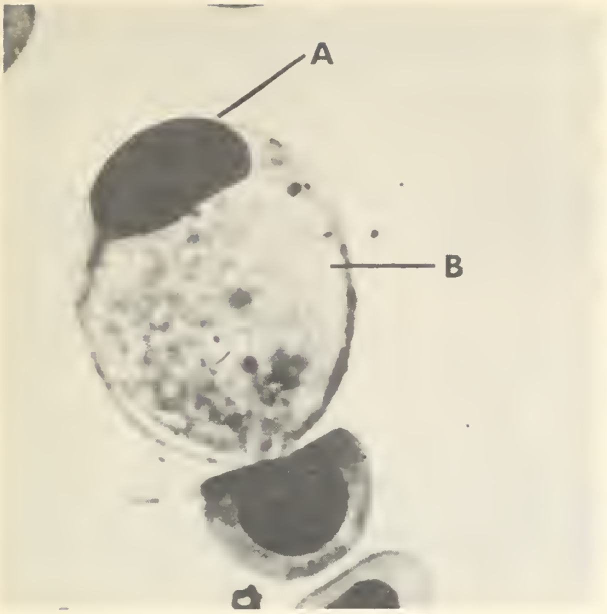

Figure 5.--A macrogametocyte of stage IV: A, host cell nucleus: B. parasite.

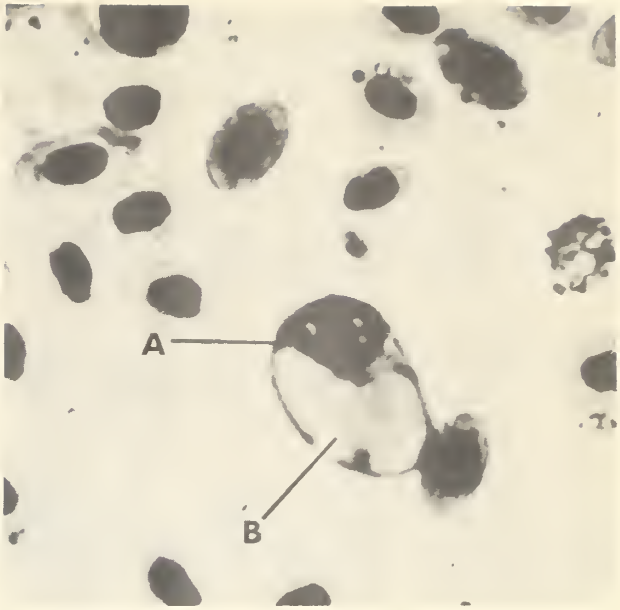

Figure 6.--A microgametocyte of stage IV: A, host cell; B, parasite.

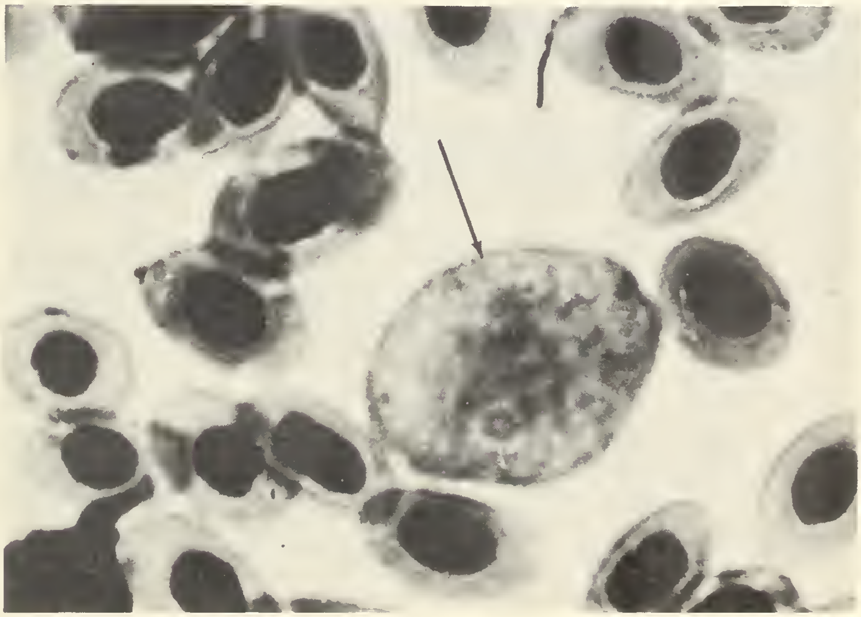

Figure 7.--A free form of macrogametocyte in stage V.

In controlled experiments with L. andrewsi, species of the Anopheles, Aedes, and Culex mosquitoes apparently were not infected after having fed on parasitized chickens (8).

In Canada wild birds were suspected of being the reservoir of L. simondii and L. smithi infections (25).

We obviously have many potential intermediate hosts and possible reservoirs of L. caulleryi in North America. The identity of the bloodsucking insects that will transmit it and will not transmit it is not known at the present time.

SIGNS OR SYMPTOMS

Poultry of all ages are susceptible to this parasite. In Japan the largest outbreaks occur in July and August every year and a few cases appear up to January (4). The disease is acute. Birds often die without showing any signs of sickness, or they die within 12 hours after appearing sick (4, 15).

Birds may appear droopy and move about with difficulty. Some may exhibit rapid and labored breathing (20), expel blood-stained ropy mucous from the respiratory tract, or pass blood per the cloaca (4, 15, 20). Many birds collapse suddenly, hemorrhage from the mouth, and die after convulsions, which last only a few minutes (4).

The mortality rate is frequently about 20 percent (15), but may go up to 90 percent (20) in affected flocks. In Japan, Akiba (4) reports the mortality rate in month-old birds is 50 to 80 percent and in adult birds, 5 to 13.3 percent.

Adult birds usually develop anemia, greenish-yellow diarrhea, and reduced or suspended egg production, which returns slowly (4).

PATHOLOGY

At necropsy, comb, wattles, visible membranes, subcutaneous tissue, and muscles appear paler than normal. The blood is watery and remains fluid. Hemorrhages are found in the lungs and kidneys. There may be gross hemorrhage from the kidneys into the peritoneal cavity. In addition to hemorrhages, megaloschizonts (grayish-white structures) are scattered throughout the lungs and kidneys as well as in the liver, spleen, pancreas, ovaries, and testes; on the mucous membranes of the alimentary and respiratory tracts; on all the serous membranes and the meninges; and in the subcutaneous fascia (4, 15, 20).

In the visceral organs, megaloschizonts frequently occur as scattered individual bodies measuring up to 300 μ in diameter. Each megaloschizont is therefore just visible to the naked eye. Megaloschizonts also occur in groups in the visceral organs (fig. 2). However, the tendency toward group formations of megaloschizonts is more pronounced on the mucous and serous membranes. A group of megaloschizonts is frequently oval in shape and may reach 1.5 mm. to 2 mm. or more in size. The group frequently has a variegated white and red appearance, which results from the entry of blood into the megaloschizonts that have discharged their merozoites (15).

Histopathological examination will reveal liver necrosis and lymphocytic infiltration. Megaloschizonts in all of the organs are encapsulated by a thick hyalinous wall (4).

DIAGNOSIS

Three fairly simple methods can be used in the laboratory to check for the presence of Leucocytozoon in poultry.

1. Depending upon the stage of the infection when examination is made, blood smears stained with a Romanowski or Giemsa stain, show one or more of the following:

   (a) merozoites in the plasma (fig. 3),
   (b) merozoites in the erythrocytes (fig. 4),
   (c) developing gametocytes in immature and mature erythrocytes, and
   (d) intracellular and extracellular mature gametocytes (figs. 5, 6, and 7).

The gametocytes of L. caulleryi are round or oval in shape, and when developing in the host cell, cause a round distortion of the cell (figs. 5, 6, and 7) in contrast to the spindle-shaped distortion that occurs with other species of Leucocytozoon.

2. Diagnosis depends upon finding the stages listed above. It is unnecessary to section tissues to discover megaloschizonts in the tissues. Crush preparations, made by crushing small portions of lung, kidney, or other tissues between two glass slides, serve adequately for detection of megaloschizonts by naked eye or for observation of them under low-power magnification (15).

3. Stage III of the parasite included in the host cell, as described in the gametogony phase of the life cycle, is easily seen in impression smears of the organs. It is observed less frequently in the blood films, in which the majority of the parasites are at stage II (22).

Bennett (9) has described another procedure that can be used if the proper equipment is available. He states that heavy and medium infections of hematozoa are readily detected by the examination of dried, stained blood films, fresh drops of blood, or fresh bone marrow preparations. However detection of light parasitaemia is tedious and time consuming and may give false negative results.

When small samples of avian blood in hematocrit capillary tubes are centrifuged, trypanosomes and microfilariae concentrate and are readily seen, even in low parasitaemia, in the serum above the buffy layer. Stained smears of the buffy layer and top 2 to 3 mm. of the red cell layer from the capillary tubes will show high concentrations of gametocytes of Leucocytozoon and of Haemoproteus.

DIFFERENTIAL DIAGNOSIS

The field worker should be careful not to confuse leucocytozoonosis with fowl cholera. The small grayish-white foci in the liver resemble superficially the hepatic lesions of fowl cholera, but a crush preparation will show megaloschizonts in cases of leucocytozoonosis; blood smears will reveal the absence of Pasturella multocida (15).

When groups of megaloschizonts are found under the serosa of the intestine, and especially when hemorrhage has occurred into ruptured megaloschizonts, the intestinal lesions may resemble those caused by Eimeria necatrix. Examination of the lesions with a hand lens will, however, reveal the presence of megaloschizonts (15).

The acuteness of leucocytozoonosis, and the expulsion of bloody mucous from the mouth may lead one to suspect laryngotracheitis when observing a sick flock. However, a necropsy and one of the previously described laboratory procedures should provide the diagnostician with a quick differential diagnosis between these two diseases.

CONTROL AND TREATMENT

Leucocytozoonosis in turkeys and ducks is controlled by screening the intermediate hosts away from the young poults and ducklings. It appears that the task of controlling L. caulleryi may be more difficult because adult birds are quite susceptible to infection, and the intermediate host may be too small for screening out of houses. No drug has yet been found that has any action on stages of the parasite in avian hosts.

The control of Culicoides whose breeding stages occur extensively in paddy fields and marshes is not yet feasible (15).

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